IL-37- and IL-35/IL-37-Producing Plasma Cells in Chronic Periodontitis

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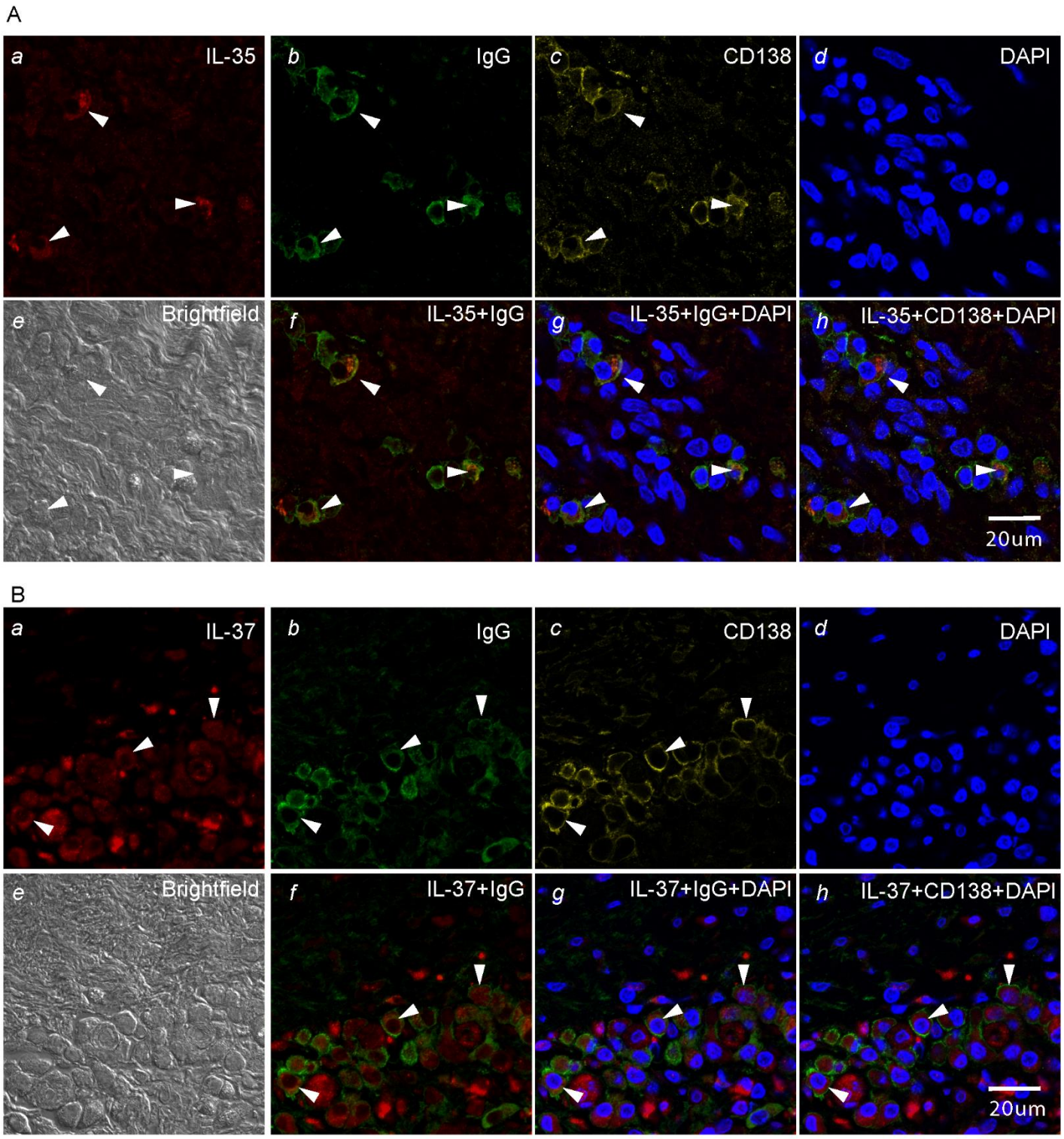
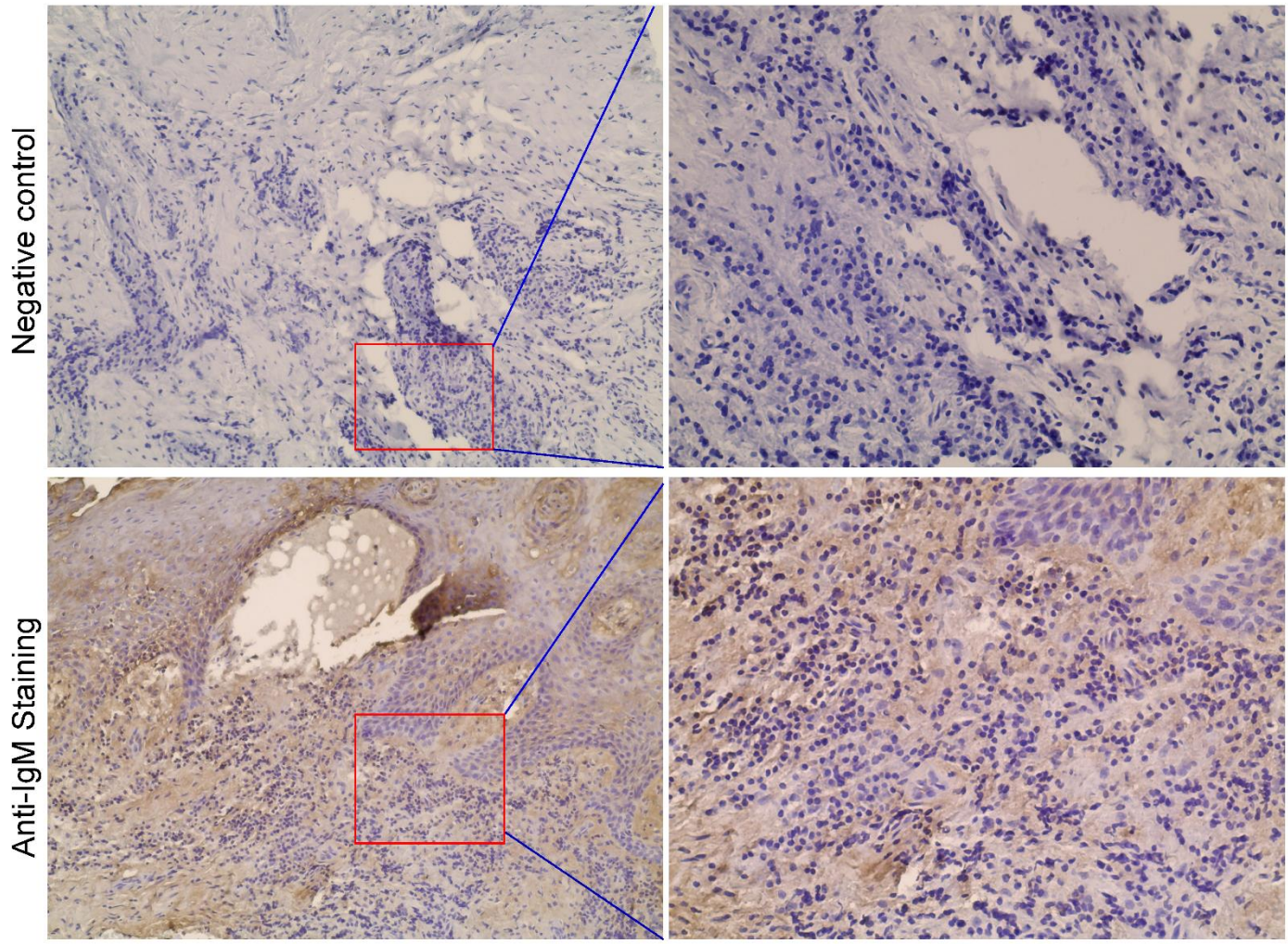
Appendix



Supplementary Figure 1: Co-localization of plasma cell marker (CD138), IgG, IL-35 or IL-37 in gingival tissue from periodontitis patients.

A: The co-localization of IL-35 and IgG in human gingival tissue was performed using immunofluorescence and confocal microscopy. **Panel *a-e*:** show the single layer of CD138, IgG, IL-35, DAPI and Bright field in human gingival tissue. **Panel *f-h*:** show the merged layers of IL-35&IgG, IL-35&IgG&DAPI and IL-35&CD138&DAPI. Arrows indicate some of the representative IgG^+ i35-Pregs in the slides.

B: The co-localization of IL-37 and IgG in human gingival tissue was performed using immunofluorescence and confocal microscopy. **Panel *a-e*:** show the single layer of CD138, IgG, IL-37, DAPI and Bright field. **Panel *f-h*:** show the merged layers of IL-37&IgG, IL-37&IgG&DAPI and IL-37&CD138&DAPI. Arrows indicate some of the representative IgG^{+} i37-Pregs in the slides. Scale bars are shown in the panel.



Supplementary Figure 2: Expression level of IgM in human gingival tissues.

The expression of IgM was characterized using anti-human IgM specific antibody by IHC. Result are shown as the representative DAB staining of IgM in CP lesions.

The Inclusion and Exclusion Criteria of Gingival Tissue Biopsies:

In detail, the inclusion criteria included: 1: Adults over 35 years old. 2: Able to give consent to participate in research. 3: At least 20 teeth. 4: Good general health as evidenced in their medical history. 5: Having at least 6 teeth with pocket depth (PD) >4 mm and clinical attachment level (CAL) >2 mm. Major exclusion criteria included: 1: Receipt of antibiotic-related therapy for medical or dental reasons 3 months prior to study inclusion. 2: Long-term use of medications known to affect periodontal status such as anti-inflammatory drugs, aspirin and ibuprofen. 3: Use of immunosuppressive therapies, including glucocorticoids or cyclosporines. 4: History of smoking. 5: Any systemic condition that requires antibiotic coverage for periodontal procedures (e.g. heart conditions, joint replacement).

Supplementary Table 1: Summary of primary antibodies used in this study.

Primary antibody	Host	Antigen retrieval	Working dilution	Source
IL-35	Mouse	Sodium Citrate buffer PH6.0	1:70	Novus Biologicals; IL-27/IL-35 EBI3 Subunit Monoclonal Antibody; Catalog #:NBP2-27362
IL-37	Rabbit	Sodium Citrate buffer PH6.0	1:1000	Thermo Fisher; IL-1F7 Polyclonal Antibody; Catalog #:PA5-30527
CD138	Goat	Sodium Citrate buffer PH6.0	1:150	R&D; Human Syndecan-1/CD138 Polyclonal Antibody; Catalog #:AF2780
CD38	Rabbit	Sodium Citrate buffer PH6.0	1:50	MyBioSource; Human CD38 Polyclonal Antibody; Catalog #: MBS302125
IgG	Rabbit	Sodium Citrate buffer PH6.0	1:70	Thermo Fisher; Human IgG Fc Polyclonal Antibody, FITC; Catalog #:31535
IgM	Rabbit	Sodium Citrate buffer PH6.0	1:1000	Novus Biologicals; Human IgM Polyclonal Antibody; NB120-17150

Supplementary Table 2: Summary of Fluorescence conjugated secondary antibodies used in this study.

Secondary antibody	Fluorescence color	Working dilution	Source
Cy5 Donkey Anti-Rabbit IgG	Cy5 (far red)	1:500	Jackson ImmunoResearch; Catalog #711-175-152
Cy3 Donkey Anti-Goat IgG	Cy3 (orange)	1:500	Abcam; Catalog #:ab6949
Alexa Fluor 488 Donkey anti-goat IgG	Alexa Fluor 488 (green)	1:500	Jackson ImmunoResearch; Catalog #705-545-147
Alexa Fluor 647 Goat anti-Mouse IgG	Alexa Fluor 647 (far red)	1:500	Abcam; Catalog #:ab150115
Cy3 Goat anti-Mouse IgG	Cy3 (orange)	1:500	Jackson ImmunoResearch; Catalog #115-165-146

Supplementary Table 3: Combination of secondary antibodies for co-localization staining

Co-localization	Secondary antibody
IL-35 & CD138	Alexa Fluor 488 Donkey anti-goat (for CD138) & Alexa Fluor 647 Goat anti-Mouse IgG (for IL-35)
IL-35 & CD138 & CD38	Alexa Fluor 488 Donkey anti-goat (for CD138) & Cy3 Goat anti-Mouse IgG(for IL-35) & Cy5 Donkey Anti-Rabbit IgG (for CD38)
IL-37 & CD138	Cy5 Donkey Anti-Rabbit IgG(for IL-37) & Alexa Fluor 488 Donkey anti-goat IgG (for CD138)
IL-35 & IL-37 & CD138	Cy5 Donkey Anti-Rabbit IgG (for IL-37) & Alexa Fluor 488 Donkey anti-goat IgG (for CD138) & Cy3 Goat anti-Mouse IgG (for IL-35)
IL-35 & IgG & CD138	Alexa Fluor 647 Goat anti-Mouse IgG (for IL-35) & Anti-human IgG primary antibody (FITC) & Cy3 Donkey Anti-Goat IgG (for CD138)
IL-37 & IgG & CD138	Cy5 Donkey Anti-Rabbit IgG (for IL-37) & Anti-human IgG primary antibody (FITC) & Cy3 Donkey Anti-Goat IgG (for CD138)